(c) purifying the DNA from the remainder of the biological material; and

(d) analyzing the purified DNA,

wherein the lysing reagent is bound to the solid support and any unbound lysing reagent is removed from the solid support before the biological material is contacted with the solid support, wherein the lying reagent essentially comprises low concentrations of reagents selected from the group of reagents consisting of buffers, salts, acids, bases, chelating agents, and detergents.

2. (**Amended**) A process for characterizing DNA comprising the step of isolating nucleic acids, wherein the step of isolating comprises the steps of:

(a) contacting a biological material that contains DNA with a solid support treated with a lysing reagent wherein the solid support has not contacted the biological material at the time of treatment;

(b) treating the biological material that contains DNA with a DNA purifying reagent;

(c) applying a DNA eluting reagent to the solid support; and

(d) purifying the DNA from the remainder of the biological material,

wherein the DNA eluting reagent comprises:

(i) a buffer;

(ii) a base;

(iii) a chelating agent; and

(iv) water,

wherein the lysing reagent is bound to the solid support and any unbound lysing reagent is removed from the solid support before the biological material is contacted with the solid support, wherein the lying reagent essentially comprises low concentrations of reagents selected from the group of reagents consisting of buffers, salts, acids, bases, chelating agents, and detergents.

3. (**Previously Presented**) The process of claims 1 or 2, wherein the solid support is contained in a vessel, wherein the vessel is selected from a group consisting of centrifuge

tubes, spin tubes, syringes, cartridges, chambers, multiple-well plates, test tubes, and combinations thereof.

4. **(Previously Presented)** The process according to claims 1 or 2, comprising the further step of heating the solid support to greater than 60°C.

5. **(Previously Presented)** The method of claims 1 or 2, wherein the biological material is selected from the group consisting of eukaryotic cells, prokaryotic cells, microbial cells, bacterial cells, plant cells, mycoplasma, protozoa, [bacteria,] fungi, viruses, and lysates and homogenates thereof.

6. **(Previously Presented)** The method of claims 1 or 2, wherein the biological material is selected from the group consisting of body fluids, body waste products, excretions, and tissues.

7. **(Previously Presented)** The method of claims 1 or 2, wherein the biological material is an environmental sample taken from air, water, sediment or soil.

8. **(Previously Presented)** The process according to claim 5, further comprising the step of counting eukaryotic cells when the biological material is eukaryotic cells.

9. **(Previously Presented)** The process according to claim 5, further comprising the step of counting prokaryotic cells when the biological material is prokaryotic cells.

10. **(Previously Presented)** The process according to claim 5, further comprising the step of counting viruses when the biological material is viruses.

12. **(Previously Presented)** The process according to claims 1 or 2, wherein the isolating step further comprises the step of analyzing the remainder of the biological material.

13. **(Amended)** The process according to claim ~~11~~ 12, wherein the analyzing step further comprises the step of monitoring impurities.

14. **(Previously Presented)** The process according to claim 12, wherein the analyzing step further comprises the step of monitoring impurities.

15. **(Previously Presented)** The process according to claims 1 or 2, further comprising the step of quantitating the purified DNA.

16. **(Previously Presented)** The process according to claims 1 or 2, further comprising the step of adjusting the concentration of DNA.

17. **(Previously Presented)** The process according to claims 1 or 2, further comprising the step of evaluating the purified DNA.

18. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprises the step of determining the yield of purified DNA.

19. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprises the step of determining the size of the purified DNA or fragments thereof.

20. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprisesa step of determining the purity of DNA.

21. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprises a step of digesting the purified DNA with a restriction enzyme or other DNA modifying enzyme.

22. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprises a step of analyzing the sequence of the purified DNA.

23. **(Previously Presented)** The process according to claim 17, wherein the step of evaluating the purified DNA further comprises a step of conducting a hybridization analysis on the purified DNA.

24. **(Previously Presented)** The process according to claim 1, further comprising a step of amplifying the purified DNA.

25. **(Previously Presented)** The process according to claim 2, further comprising a step of amplifying the purified DNA.

26. **(Amended)** A process for amplifying DNA sequences, wherein the process comprises the steps of:

(a) contacting a biological material that contains DNA with a solid support treated with a lysing reagent wherein the solid support has not contacted the biological material at the time of treatment;

(b) treating the biological material with a DNA purifying reagent;

(c) purifying the DNA; and

applying the purified DNA to an amplification system, wherein the lysing reagent is bound to the solid support and any unbound lysing reagent is removed from the solid support before the biological material is contacted with the solid support~~.~~, wherein the lying reagent essentially comprises low concentrations of reagents selected from the group of reagents consisting of buffers, salts, acids, bases, chelating agents, and detergents.

27. **(Amended)** A process for amplifying DNA sequences, wherein the process comprises the steps of:

(a) contacting a biological material that contains DNA with a solid support reated with a lysing reagent wherein the solid support has not contacted the biological material at the time of treatment;

(b) treating the biological material with a DNA purifying reagent;

(c) applying a DNA eluting reagent to the solid support;

(d) purifying the DNA; and

(e) applying the purified DNA to an amplification system,

wherein the DNA eluting reagent comprises:

(i) a buffer;

(ii) a base;

(iii) a chelating agent; and

(iv) water.

, wherein the lying reagent essentially comprises low concentrations of reagents selected from the group of reagents consisting of buffers, salts, acids, bases, chelating agents, and detergents.

28. **(Previously Presented)** The process of claims 26 or 27, wherein the solid support is contained in a vessel, wherein the vessel is selected from the group consisting of centrifuge tubes, spin tubes, syringes, cartridges, chambers, multiple-well plates, test tubes, and combinations thereof.

29. **(Previously Presented)** The process of claims 26 or 27, wherein the biological material is selected from the group consisting of eukaryotic cells, prokaryotic cells, microbial cells, bacterial cells, plant cells, mycoplasma, protozoa, fungi, viruses, and lysates and homogenates thereof.

30. **(Previously Presented)** The process of claim 26 or 27, wherein the biological material is selected from the group consisting of body fluids, body waste products, excretions, and tissues.

31. (**Previously Presented**) The method of claim 26 or 27, wherein the biological material is an environmental sample taken from air, water, sediment or soil.

32. (**Previously Presented**) The process of claims 26 or 27, wherein the biological material is applied to the solid support without any prior treatment of the biological material.

33. (**Previously Presented**) The process of claims 26 or 27, wherein the solid support is selected from the group consisting of cellulose, cellulose acetate, glass fiber, nitrocellulose, nylon, polyester, polyethersulfone, polyolefin, polyvinylidene fluoride, and combinations thereof.

34. (**Previously Presented**) The process of claim 33, wherein the polyolefin is a mixture of low density polyethylene and polypropylene fibers.

35. (**Previously Presented**) The process of claim 33, wherein the polyolefin is hydrophilic.

37. (**Previously Presented**) The process of claim 33, wherein the lysing reagent comprises:
(a) a detergent effective to lyse the biological material sufficiently to release DNA;
(b) water; and optionally
(c) an RNA digesting enzyme.

38. (**Previously Presented**) The process of claim 33, wherein the lysing reagent comprises:
(a) a detergent effective to lyse the biological material sufficiently to release DNA;
(b) water; and optionally
(c) an RNA digesting enzyme; but
(d) does not contain a buffer.

39. (**Previously Presented**) The process of claim 33, wherein the lysing reagent comprises:
(a) a detergent effective to lyse the biological material sufficiently to release DNA;
(b) water; and optionally

(c) an RNA digesting enzyme; but

(d) does not contain a chelating agent.

40. (**Previously Presented**) The process of claim 33, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) a chelating agent to reduce damage to DNA;

(c) water; and optionally

(d) an RNA digesting enzyme; but

(e) does not contain a buffer.

41. (**Previously Presented**) The process of claim 33, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) a buffer;

(c) water; and optionally

(d) an RNA digesting enzyme; but

(e) does not contain a chelating agent.

42. (**Previously Presented**) The process of claim 27, wherein the DNA eluting reagent has a pH of at least about 10, and the combined concentration of buffer, base, and chelating agent is no greater than about 20 mM, based on the total volume of the DNA eluting reagent.

43. (**Previously Presented**) The process of claim 27, wherein the DNA eluting reagent has a pH of at least about 9, and the combined concentration of buffer, base, and chelating agent is no greater than about 20 mM, based on the total volume of the DNA eluting reagent.

44. (**Previously Presented**) The process of claims 26 or 27, further comprising the step of heating at greater than 60°C.

45. **(Previously Presented)** The process of claims 24 or 25, further comprising the step of amplifying using an amplification system.

46. **(Previously Presented)** The process of claim 26, 27, or 45, wherein the amplification system comprises buffer, primers, deoxyribonucleotides, a thermostable DNA polymerase, and a programmable heating element.

47. **(Previously Presented)** The process of claims 26, 27, or 45, further comprising the step of quantitating the amplified DNA.

48. **(Previously Presented)** The process of claims 26, 27, or 45, further comprising the step of evaluating the amplified DNA.

49. **(Previously Presented)** The process of claim 48, wherein the step of evaluating the amplified DNA further comprises a step of determining the size of the amplified DNA.

50. **(Previously Presented)** The process of claim 48, wherein the step of evaluating the amplified DNA further comprises a step of digesting the amplified DNA with a restriction enzyme.

51. **(Previously Presented)** The process according to claim 48, wherein the step of evaluating the amplified DNA further comprises a step of sequencing the amplified DNA.

52. **(Previously Presented)** The process according to claim 48, wherein the step of evaluating the amplified DNA further comprises a step of analyzing the sequence of the amplified DNA.

53. **(Previously Presented)** The process according to claim 48, wherein the step of evaluating the amplified DNA further comprises the step of conducting a hybridization analysis on the amplified DNA

54. **(Previously Presented)** A process for analyzing DNA comprising a step of isolating nucleic acids, wherein the step of isolating comprises the steps of:

(a) contacting a biological material that contains DNA with a solid support treated with a lysing reagent wherein the solid support has not contacted the biological material at the time of treatment;

(b) heating the solid support;

(c) treating the biological material that contains DNA with a DNA purifying reagent;

(d) purifying the DNA from the remainder of the biological material; and

(e) analyzing the purified DNA;

wherein the lysing reagent is bound to the solid support; wherein the lysing reagent is bound to the solid support and dried to the solid support.

55. **(Previously Presented)** A process for amplifying DNA sequences, wherein the process comprises the steps of:

(a) contacting a biological material that contains DNA with a solid support treated with a lysing reagent wherein the solid support has not contacted the biological material at the time of treatment;

(b) treating the biological material with a DNA purifying reagent;

(c) purifying the DNA; and

(d) applying the purified DNA to an amplification system,

wherein the lysing reagent is bound to the solid support and dried to the solid support.

56. **(Previously Presented)** The process of claim 1, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) water; and optionally

(c) an RNA digesting enzyme.

57. **(Previously Presented)** The process of claim 1, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) water; and optionally

(c) an RNA digesting enzyme; but

(d) does not contain a buffer.

58. **(Previously Presented)** The process of claim 1, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) water; and optionally

(c) an RNA digesting enzyme; but

(d) does not contain a chelating agent.

59. **(Previously Presented)** The process of claim 1, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) a chelating agent to reduce damage to DNA;

(c) water; and optionally

(d) an RNA digesting enzyme; but

(d) does not contain a buffer.

60. **(Previously Presented)** The process of claim 1, wherein the lysing reagent comprises:

(a) a detergent effective to lyse the biological material sufficiently to release DNA;

(b) a buffer;

(c) water; and optionally

(d) an RNA digesting enzyme; but

(e) does not contain a chelating agent.

61. **(Previously Presented)** The process of claim 1, wherein the lysing reagent is anionic.

62. **(Previously Presented)** The process of claim 26, wherein the lysing reagent is anionic.

CLAIM OBJECTIONS

Item 4. The Examiner states that "[c]laim 13 is objected to because of the following informalities: Claim 13 depends upon Claim 11 which has been cancelled. Appropriate correction is required."

It is respectfully pointed out to the Examiner that Claim 13 has been corrected.

CLAIM REJECTIONS - 35 USC 102

Item 5. The Examiner states that "[c]laims 1, 3-6, 15-17, 22-24, 26, 28-30, 32-33, 37-38, 41, 44-49, 53, 54,58, 60 are rejected under 35 U.S.C. 102(e) as being anticipated by Harvey et al. (US Pat. 5,939,259, August 1999)."

It is respectfully pointed out to the Examiner that the reagents of the present invention contain low concentrations of buffers, salts, acids, bases, chelating agents, and/or detergents so that they are not significantly inhibitory to subsequent DNA analyses. In conventional systems, reagents containing high concentrations of one or more of these components are typically used for DNA purification. These reagents are also generally less hazardous than those used for conventional DNA purification. See Page 2 of the instant specification. Specifically, the reagents used in the lysing reagent of the present invention are less hazardous than those contemplated by Harvey. Harvey utilizes conventional chaotropic salts such as guanidine isothiocyanate, guanidine thiocyanate, guanidine hydrochloride, sodium iodide, sodium perchlorate, potassium iodide, sodium (iso) thiocyanate, urea, or combinations thereof. See Harvey, Col. 3, Lines 27-31. Lysis of cells by hazardous, high concentration chaotropic reagents is known to those skilled in the art. However, lysis of cells by immobilized lysis reagents essentially comprising low concentrations of buffers, salts, acids, bases, chelating agents, and/or detergents is a novel teaching of the present invention. The Examiner is reminded that substances such as guanidium and urea must be used at such high concentrations as 6M in solution to cause lysis. When, however, these substances are used at very low concentrations, they tend to cause cell adhesion and binding, and are often components of cell culture solutions. Furthermore, it is respectfully pointed out to the Examiner that nowhere does Harvey teach that

the immobilized concentration of chaotropes as used in the invention cause complete lysis of the cells. Harvey merely teaches that nucleic acids released after washing the membrane may be amplified. A very small amount of nucleic acids are required for amplification methods known to those skilled in the art. Thus, the claims, as amended, distinguish the teachings of the present invention from that of Harvey.

CLAIM REJECTIONS - 35 USC 103

Item 6. The Examiner states that "[c]laims 1-3, 5-6, 11-21, 23-30, 32-33, 37, 39, 41, 45-51, 53-56, 58, 60-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom et al (5,234,809) in view of Shieh (US Pat. 6,054,039, April 2000)."

The Examiner rebutted the arguments filed on June 6, 2003. The applicant wishes to maintain the arguments stated in the response filed on June 6, 2003.

The applicant wishes to reiterate that Boom teaches that "it is essential to use a chaotropic substance" such as guanidinium (iso) thiocyanate and guanidinium hydrochloride, and urea. See Boom, Col. 3, lines 56-67. Also, see Boom, Claim 3. Thus, the process according to Boom requires the use of highly toxic chaotropic substances such as the aforementioned chaotropes. The claims of the present invention, as amended, are directed to lysing reagents essentially comprising low concentrations of buffers, salts, acids, bases, chelating agents, and/or detergentst. Indeed, it is stated in the specification that the invention seeks to avoid the use of hazardous substances such as those taught in Boom.

The Examiner states that Shieh is being relied upon [in this 103 rejection] for the teachings that lysing reagents, generally, may be dried upon a solid support and allow lysis. The Examiner also goes on to state that the ordinary artisan would have been motivated to have prepared the pre-treated lysing membrane of Shieh, for use in the method of Boom, and that the skilled artisan would have had a reasonable expectation of success for analyzing DNA from a solid support that was pretreated with a lysing reagent since Boom teaches a method in which all

three components, a lysing reagent, solid support and nucleic acid sample, were contacted with successful results.

It is respectfully pointed out to the Examiner, that according to the method disclosed in Boom, "it is essential to use a chaotropic substance" such as guanidinium (iso) thiocyanate and guanidinium hydrochloride, and urea. See Boom, Col. 3, lines 56-67. Also, see Boom, Claim 3. Thus, the process according to Boom requires the use of highly toxic chaotropic substances such as the aforementioned chaotropes. Sufficiently large amounts of chaotropes are mixed with the biological material (for example in a chaotrope:biological material ratio of 1:18). Thus, although Boom teaches the combination of a lysing reagent (chaotrope), solid support (beads) and nucleic acid sample, Boom specifically teaches the combination of an excess of chaotropic lysing reagent to enable complete lysis of the biological sample. One skilled in the art could not be motivated to characterize DNA as taught in the instant invention by immobilizing the chaotropic reagents as taught by Boom on the membrane of Shieh. The fact that Boom requires a chaotrope in excess to cause lysis teaches away from applying a very small fractional amount of that lysing reagent to a membrane to cause lysis.

Item 7. The Examiner states that "[c]laims 1-20, 24-33, 37-41, 44-49, 54-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000)."

It is respectfully pointed out to the Examiner that Deggerdal does not teach an invention in which lysing reagent is bound to the solid support and any unbound lysing reagent is removed from the solid support before the biological material is contacted with the solid support. Deggerdal teaches the same method as Boom does in which the solid support, biological material and lysing reagent are mixed together such that there is an excess of lysing reagent to assist in lysing. Thus, Deggerdal does not teach a fundamental aspect of the present invention. One skilled in the art could not be motivated to apply the teaching of Deggerdal to that of Shieh because Deggerdal specifically teaches lysis in an excess of lysing reagent in a suspension of biological material, membrane, and lysing reagent.

Item 8. The Examiner states that "[c]laims 38 and 40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,234,809) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-3, 5-6, 11-21, 23-30, 32-33, 37, 39, 41, 45-51, 53-56, 58, 60-62 above, and further in view of in view of Deggerdal (WO 96/18731)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Item 9. The Examiner states that "[c]laims 23 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-20, 24-33, 37-41, 44-49, 54-62 above and further in view of Boom (5,234,809)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Deggerdal, Boom and Shieh overcome this rejection cited by the Examiner.

Item 10. The Examiner states that "[c]laims 7, 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,234,809) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-3, 5-6, 11-21, 23-30, 32-33, 37, 39, 41, 45-51, 53-56, 58, 60-62 above and further in view of Su (5,804,684)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Item 11. The Examiner states that "[c]laims 42-43 are rejected under 35 U.S.C. 104(a) as being unpatentable over Boom (5,804,684) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-3, 5-6, 11-21, 23-30, 32-33, 37, 39, 41, 45-51, 53-56, 58, 60-62 above or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-20, 24-33, 37-41, 44-49, 54-62 above and further in view of Su (5,804684)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Item 12. The Examiner states that "[c]laims 22 and 51-52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,804,684) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-3, 5-6, 11-21, 23-30, 32-22, 37, 39, 41, 45-51, 53-56, 58, 60-62 above or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039), April 2000) as applied to Claims 1-20, 24-33, 37-41, 44-49, 54-62 above and further in view of Sambrook (Molecular Cloning)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Item 13. The Examiner states that "[c]laims 33 and 35-36 are rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,804,684) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-3, 5-6, 11-21, 23-30, 32-33, 37, 39, 41, 45-51, 53-56, 58, 60-62 above or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) as applied to Claims 1-20, 24-33, 37-41, 44-49, 54-62 above and further in view of Arnold (5,599,667)."

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Item 14. The Examiner states that "[c]laims 34 is rejected under 35 U.S.C. 103(a) as being unpatentable over Boom (5,804,684) in view of Shieh (US Pat. 6,054,039, April 2000) or Deggerdal (WO 96/18731) in view of Shieh (US Pat. 6,054,039, April 2000) and further in view of Arnold (5,599,6667) as applied to claim 33, 35-36 above, and further in view of Hasebe (5,151,345).

The aforementioned discussions detailing the differences between the instant invention and the inventions of Boom and Shieh overcome this rejection cited by the Examiner.

Based on the amendments and remarks above, applicants believe that all pending claims are in condition for allowance.

If the Examiner believes that a conference would be of value in expediting the prosecution of this application, the Examiner is hereby invited to telephone undersigned counsel to arrange for such a conference.

Respectfully submitted,

Dated: March 9, 2004

Sanjay Sitlani
Reg. No. 48,489
Ropes & Gray
One Metro Center
700 12th Street, N.W.
Suite 900
Washington DC 20006